

A Review on Effect of Antitubercular Activity on Structural Modification of Isoniazide

Mohammad Asif*

Department of Pharmacy, GRD(PG)IMT, Dehradun, 248009, (Uttarakhand), India.

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Abstract: Tuberculosis remains the leading cause of mortality worldwide. The facts relating to a front-line antitubercular drug isoniazide and its analogues. The antitubercular pharmacophore moiety of isoniazide has been introduced in various compounds to improve their antitubercular activity against mycobacterium, as well as their multidrug resistant mycobacterium strains. Various compounds like Schiff bases, hydrazones, hydrazides and metal complexes of isoniazide have shown good antitubercular activity. A series of isoniazid analogues have been evaluated for their activity against *Mycobacterium tuberculosis*. Some compounds exhibited significant antitubercular activity when compared with first line drugs like isoniazid and rifampicin and could be a good starting spot to develop new compounds in the fight against multi-drug resistant tuberculosis.

Keywords: Isoniazide analogs, M. tuberculosis, multidrug-resistance, anti-tubercular agents.

1 Introduction

Mycobacterium tuberculosis (*Mtb*), the contributing agent of tuberculosis (TB), is a highly contagious bacterial disease that has killed about one billion people over the last two centuries and has responsible for one-seventh of all deaths globally. Pulmonary TB or “the white plague,” is the primary illness caused by *Mtb*. The *Mtb* bacilli were first identified by Robert Koch in 1882. A killed-bacterium vaccine (Bacille Camille-Guerrin or BCG) became available in 1924 and effective antibiotics against *Mtb* were developed in the 1940s. Despite these advances, deaths from *Mtb* infection continue to total nearly three million people per year worldwide. The World Health Organization (WHO) has estimated that up to a third of the world population is currently infected with *Mtb* strains (World Health Organization. 1998).

The compromised immune status of individuals infected with human immunodeficiency virus (HIV) increased the incidence of reactivation of latent *Mtb* infection. As infections with HIV have continued to proliferate, a concurrent rise in the number of TB cases has also occurred worldwide (Centers for Disease Control. 2000). In conjunction with the increased worldwide incidence of active TB cases, there has been an emergence of *Mtb* isolates resistant to previously effective anti-TB agents. The increased resistance has been due to several factors, primary among these the inadequate treatment of *Mtb* infections with anti-TB agents. Patients have

contributed to this problem by not taking the drugs properly or by not finishing the entire course of therapy. In both instances, susceptible organisms are killed, while resistant organisms escape killing and may proliferate. To further compound the problem, there are presently no effective means for confirming complete eradication of *Mtb* organisms from infected patient. In many cases, therapy may have abrogated the symptoms and signs but not fully eliminated the organism. *Mtb* infections might then erroneously be considered resolved, since evidence of infection is not detectable by laboratory tests. As the immune system becomes compromised, often due to aging, AIDS, or anti-cancer or anti-organ rejection therapies, anti-TB resistant *Mtb* strains begin to multiply and cause clinical symptoms and signs of active TB. Various public health organizations were unprepared for the resurgence of TB. Unlike problems presented by the TB outbreaks of the mid 1900s, health officials today are struggling to control the increasing global incidence of infection with anti-TB-resistant *Mtb*.

Tuberculosis (TB) is an important health problem particularly in people infected with HIV. Other causes that increasing TB cases are multidrug resistant strains (MDR), resulting from inconsistent or partial treatment, and the lack of new drugs in the market (Coker. 2003). Because of these problems, TB is a global health emergency. TB can mainly attack the lungs, although it can affect other organs as well.

At present, the treatment against TB involves 3 or 4 different kinds of anti-TB drugs given in combination over period of 6 to 9 months. These drugs mainly are isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB). Multiple combinations are crucial to prevent the emergence of MDR-TB organisms, which would lead to treatment failure (Frieden, and Driver. 2003; Souza. 2006; Souza, et al., 2006). In spite of TB being a world health problem, about 40 years have past since a new drug was introduced into the market. Therefore, the development of new drugs with fewer toxic side effects, improved pharmacokinetics properties, extensive and potent activity against bacteria, including resistant strains, is urgently needed. In the last few years, analogs of INH have been developed as potential anti-TB agents (Maccari, et al., 2005; Mohamad, et al., 2004; Maccari, et al., 2004; Maccari, et al., 2002; Bottari, et al., 2001; Slayden, and Barry. 2000). Various INH derivatives may enhanced activities compared INH (Neves, et al., 2005).

2 Mycobacterium Tuberculosis–Invader Of Disease

The genome sequence of *Mtb* was determined in 1998. The genome comprises 4,411,529 base pairs, contains around 4,000 genes, and has a very high guanine and cytosine content that is reflected in the biased amino-acid content of the proteins (Cole, et al., 1998). The *Mtb* differs radically from other bacteria in which a very large portion of its coding capacity is devoted to the production of enzymes involved in lipogenesis and lipolysis, and to two new families of glycine-rich proteins with a repetitive structure that may represent a source of antigenic variation. Circular map of chromosome of *Mtb* has been created. This chronic disease is caused mainly by *Mtb* and mycobacteria of the “TB complex“, including *M. bovis*, *M. africanum* and *M. microti*. *Mtb* is a slowly growing, non pigmented, non motile, non sporulating, weakly gram positive, acid-fast rod. The high lipid content of the cell wall (60% dry weight) shows a very specific characteristic. The ability to survive within phagocytes, and resistance to chemical disinfectants are important factors to be considered. It takes 2-6 weeks before colonies develop on solid media like Loevenstein-Jensen or Middlebrook substrates.

2.1 Increasing incidence of *M. tuberculosis* infection

The AIDS epidemic was directly responsible for a significant increase in TB cases and also accelerated the emergence of anti-TB-resistant bacilli. Infections with *Mtb* in HIV patients are more severe, and resistant organisms proliferate rapidly in these patients (Breathnach et al., 1998; Espinal et al., 2000; Oleksijew et al., 1998). In 1991, 90 percent of patients infected with anti-TB-resistant *Mtb* worldwide were also infected with HIV (Rastogi et al.,

1998). HIV infections are fatal in 70 to 90 percent of patients coinfecting with resistant *Mtb* (Eltringham and Drobniewski. 1998). When individuals are infected with HIV and anti-TB-resistant *Mtb*, the average patient survives for only 78 to 315 days (Eltringham and Drobniewski. 1998). In contrast, the worldwide incidence of TB has increased significantly. When the AIDS epidemic began, public health funding for TB awareness programs had been greatly reduced. In addition, many countries were socioeconomically depressed. The highest incidence of TB occurs in developing nations. Currently, 22 underdeveloped countries are responsible for 80 percent of the TB infections worldwide (Centers for Disease Control. 2000; World Health Organization. 1998; Dye et al., 1999). Coinfection with HIV and *Mtb* bacilli has placed high burdens on poor countries with inadequate public health infrastructures. The HIV epidemic has directly contributed to a 300-400 percent increase in TB cases in Africa’s sub-Saharan countries. About 32 percent of HIV patients in these countries are infected with *Mtb* (Guillemin et al., 1998). These numbers are likely lower than actual, since reliable accounting is a problem in areas with poor public health infrastructures. Spread of *Mtb* bacilli by immigration of infected individual accounts for most of the increased incidence in more developed countries (Garcia-Garcia et al., 2000; Poss. 2000; Tupasi et al., 2000).

2.2 Emergence of antimycobacterial resistance

The leading factor in the emergence of anti-TB resistant *Mtb* is probably prior inadequate treatment with anti-TB agents (Arevalo, et al., 1996; Hersi et al., 1999). Resistant *Mtb* bacilli are rarely encountered in regions where few or no anti-TB agents have been used (Petrini and Hoffner. 1999). When anti-TB therapy is usually used, however, resistant organisms gain a competitive advantage and begin to emerge as leading virulent strains encountered in the region. A causative factor in the emergence of therapy-resistant *Mtb* is the lack of effective methods to determine if anti-TB therapy has eradicated the bacilli. Patients are considered successfully treated if no acid-fast bacilli can be detected in convalescent sputum cultures. This interpretation may be inaccurate, however, since resistant *Mtb* organisms may propagate in granulomatous lesions and then revert to dormancy. A latent infection with resistant *Mtb* may subsequently reactivate if the immune system becomes compromised. Unfortunately, adequate anti-TB therapy is also commonly unavailable in many underdeveloped countries. Most community health systems in such countries have limited capacity to satisfactorily detect and characterize *Mtb* bacilli. Individuals infected with *Mtb* may substitute these cheaper, inappropriate agents (Taylor and Suarez. 2000). Worldwide, only 60 percent of patients receiving anti-TB therapy for latent *Mtb* infection complete the prescribed treatment regimen.

2.3 Mechanisms of anti-TB resistance

A patient is infected with primary-resistant *Mtb* when the infecting bacilli are resistant to one or more anti-TB agents. Secondary or acquired resistance occurs when resistance develops during treatment of an existing previously non-resistant infection. The average rate of *Mtb* primary drug resistance to a single or multiple drugs is 10.4 percent and 0.2 percent, respectively. The average rate of acquiring resistance to one or more agents is 36 percent of all cases (WHO/IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance 1994-1997). Resistance to anti-TB agents develops when mutations occur in *Mtb* genes. *Mtb* is considered multidrug-resistant (MDR-TB) if it is resistant to at least INH and RIF. The probability of proliferation of drug resistant *Mtb* increases with treatment that employs a single anti-TB agent, malabsorption of the drugs, or inadequate treatment. *Mtb* isolates exist that are resistant to all known anti-TB agents. Researchers have described several distinct mutations that cause *Mtb* bacilli to resist the effects of anti-TB agents.

3 Isoniazide

Isoniazide (INH), pyridine-4-carboxylic acid hydrazide or isonicotinic acid hydrazide is the first line TB specific-drug that is used with other anti-TB drugs. In 1912 firstly synthesized INH without any imagination of huge importance of this drug in the treatment of TB (Baum, 2005). In 1952 INH was discovered to have considerable bactericidal activity against *Mtb*. Currently it is the prophylaxis of choice due to its low dose cost, relatively low frequency of hepatotoxicity and reasonable bioavailability. In combination with RIF and PZA it still forms the main frontline TB therapy worldwide (Whitney, and Wainberg, 2002).

3.1 Isoniazid resistance

Isoniazid is a nicotinamide derivative or isonicotinic acid hydrazide (INH). The INH inhibits enoyl-acyl carrier protein reductase, which is essential for mycolic acid biosynthesis (Sacchetti and Blandchard, 1996). The INH is taken up by *Mtb* and oxidized during catalase-peroxidase production. The oxidation changes INH into an active drug that prevents *Mtb* from forming a cell wall (Basso et al., 1998). INH-resistant *Mtb* was detected shortly after the drug became available. Since the action of INH involves different genes, several specific mutations leading to INH-resistance have been described. For example, the *Mtb* *inhA* gene encodes the NADH-dependent enoyl-acyl carrier protein reductase necessary for mycolic acid biosynthesis (Quemard et al., 1995). Mutations in *inhA* coincide with INH-resistance (Basso et al., 1998). In addition, the *katG* gene encodes a catalase-peroxidase enzyme necessary for the activation of INH (Wengenack, et al., 1997). Missense mutations in the *katG* gene also occur in most INH-resistant

Mtb isolates (Lee et al., 1997; Marttila et al., 1998; Zang et al., 1992). Rattan et al (Rattan et al., 1998) showed a 67 percent correlation between INH-resistant isolates and *katG* mutations. In addition, mutations in the *aphC* gene, which encodes alkyl hydroperoxide reductase, can cause low levels of resistance to INH (Sherman et al., 1999). Furthermore, mutations of the *glf* gene could cause low-level resistance to INH (Chen and Bishai, 1998). The *Mtb* *glf* gene encodes the Glf enzyme that catalyzes the conversion of UDP-galactofuranose, which is necessary for the biosynthesis of arabinogalactan.

3.2 Isoniazid derivatives alterations

A large number of works has been done to attain useful information about mechanism of action and resistance to currently used anti-TB agents. Isoniazid (INH) has is one of the most effective anti-TB drugs used in clinical practice. The anti-TB pharmacophore moiety of INH is introduced in a various types of compounds to form simple or complicated compounds to progress their activity against *M. tuberculosis* and their MDR-TB strains. These INH molecule alterations possessing anti-TB activity are discussed.

3.3 General Isoniazid (INH) alteration

Isoniazid (INH) can undergo some structure alterations, as it is explained. Simple alterations-aromatic ring hydrogenation, change or omission of pyridine nitrogen, *N*-quarternisation, alkylene introduction between ring and carbohydrazide moiety, substitution of the aromatic ring, substitution of one or both hydrazide nitrogens or hydrazones generation or building both nitrogens in the heterocyclic ring. All the discussed agents possess higher lipophilicity than INH (1).

4 Antitubercular Activities of Isoniazid Derivatives

These compounds are simple modifications of INH (1), which could be divided into two groups. The first class contains INH derivatives without further substitution, while the second class represents quinolines as INH derivatives. All the compounds discussed in this chapter reflected the idea that hydrazide moiety is primarily involved in the interaction with the receptor and that the aromatic pocket contributes to hydrophobicity and hydrogen bonding to the receptor. The aromatic nitrogen in pyridine ring may also facilitate the reduction of the exopyridinic conjugated double bonds by NAD(P)H-type reductases. The totally hydrogenated INH analogue 2 was prepared first. INH isosteres 3-5 with modification of aromatic ring were prepared as well. Insertion of CH₂ moiety between the aromatic ring and the hydrazide group gives alkylene homologues 6,7-derivatives of compounds 1 and 5. Nevertheless, the above mentioned compounds 2-7 as well

as doubled INH molecule **8** (Klopman, et al., 1996) showed less activity than INH or even a loss of activity (Fig. 1). Further attempts to improve the activity directed toward increasing the lipophilicity and slowing and prolonging the release of INH, because it is quite soluble in water and is rapidly metabolised.

These new derivatives **9-11** set the aim decrease of therapeutic doses, which caused less negative effect of the drugs to human body, e.g. less negative hepatic effects. Compounds **9-11** (Pasqualoto, et al., 2004) used the analogy of cycles—they became 4-substituted quinoline derivatives. The increase of lipophilicity in this series is caused by C-2 substitution (H<Me<Ph). According to results in Figure (2) the lipophilicity increase seems to cause anti-TB activity decrease. It could be concluded, that the most active compound discussed in this chapter is quinoline-4-carbohydrazide (**9**), which represents the simplest modification of INH structure. This fact confirms the structure-specificity of the activity of INH.

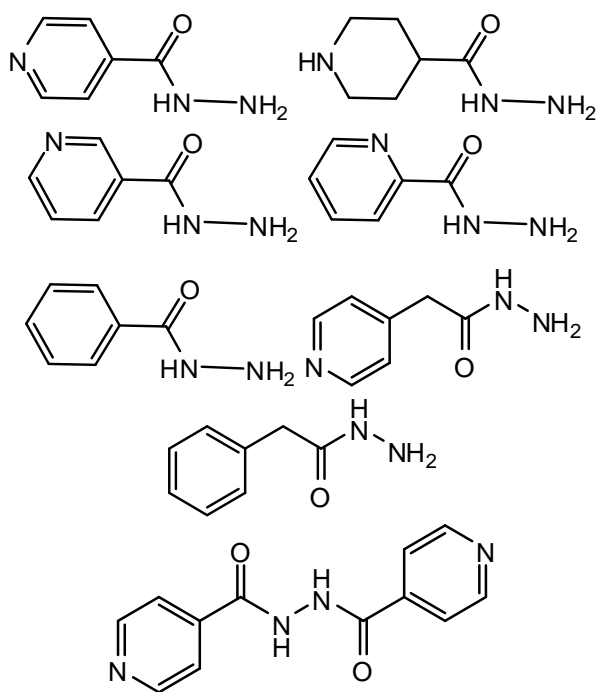


Fig. (1). Compounds 1-8, their MIC ($\mu\text{g/ml}$).

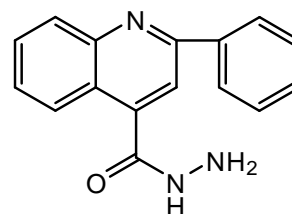
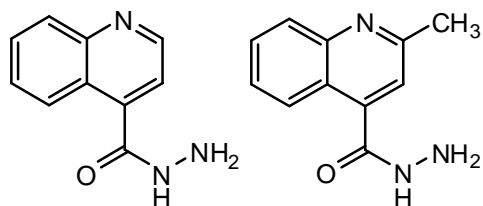


Fig. (2). Compounds 9-11, their MIC ($\mu\text{g/ml}$).

Compounds	MIC ($\mu\text{g/ml}$)	Compounds	MIC ($\mu\text{g/ml}$)	Compounds	MIC ($\mu\text{g/ml}$)
Isoniazide 1	0.02	5	0.6	9	0.079, 0.6
2	600.0	6	100.0	10	10.0, 0.79
3	2.0	7	12.0	11	>40.0, 2.13
4	0.15	8	1.0		

4.1 Substituted on C-2 Position

Substitutions in the C-2 position of pyridine ring represented the oldest INH structure modifications. A number of types of C-2 substituents have been described in literature. The most active C-2 substituted INH derivatives (Table 1). These C-2 substituted compounds include alkyl analogues **12-17**, phenyl analogues **18, 19** and isosteres of alkyl analogues: *O*-derivatives **20, 21**, *N*-derivatives **22-25**, and halogenated derivatives **26-28**. These variations involve as hydrophobic/hydrophilic as electron-donor/electron-withdrawing substituents. The activity seems to be linear decrease with lipophilicity increase (H > CH₃ > C₂H₅ > CH₂=CH₂ > *n*-C₃H₇ > *i*-C₄H₉) or decrease with substituent bulkiness (NH₂ > OCH₃ > OC₂H₅ > N(C₂H₅)₂ or F > Cl > Br). The most active 2-methylisoniazide (**12**) represents the simplest INH modification. The activity is not strongly dependent on lipophilicity (compounds **1, 12, 13, 23**). The activity is probably positively influenced by electron-donor slightly hydrophobic small bulky substituents. Compound **18** is cyclic analogue of **11** and exhibited similar anti-TB effect, which confirms the disadvantage of the bulky substituents in pyridine ring.

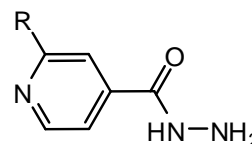


Table 1. The Most Active C-2 Substituted INH Derivatives 12-28, their MIC ($\mu\text{mol/l}$)(41) Comp. R MIC ($\mu\text{mol/l}$).

Compounds	R	MIC ($\mu\text{mol/l}$)	Compounds	R	MIC ($\mu\text{mol/l}$)
12	CH ₃	5.2	21	C ₂ H ₅ O	450.0
13	C ₂ H ₅	21.1	22	NO ₂	371.0

14	CH ₂ =CH ₂	35.0	23	NH ₂	14.5
15	<i>n</i> -C ₃ H ₇	55.2	24	(C ₂ H ₅) ₂ N	717.0
16	<i>i</i> -C ₄ H ₉	450.0	25	CH ₃ CONH	2150.0
17	CH ₃ CONHCH ₂	243.0	26	F	260.0
18	C ₆ H ₅	50.0	27	Cl	392.0
19	C ₆ H ₅ CH ₂	38.5	28	Br	616.0
20	CH ₃ O	153.0			

4.2 Hydrazone Analogues of INH

The INH derivatives describes the most active *N*(2)-mono- and *N*(2)-disubstituted hydrazides.

4.2.1 *N*-2 Monosubstituted Hydrazone Derivatives

The INH derivative substituted by ethyl in *N*-2 (**29**) is the simplest alkyl analogue of INH with acceptable anti-TB activity. Compound **29** is the model of the following hydrazone INH derivatives (Table 2). Prolongation of alkyl chain (compounds **31**, **32**) or isosteric homologues **40** and **41** cause lipophilicity increase but not significant increase of activity. Trifluoromethyl isostere **30** of compound **29** is more interesting. It possesses a similar lipophilicity (according to MDL QSAR program) and bulkiness as **29**, but showed much higher activity. This fact is probably caused by easier release of INH from the molecule, it means easier hydrolysis of the hydrazone bond between *N*-2 and trifluoroethyl moiety than the ethyl chain. The phenyl cyclic analogue **35** of the model **29** showed subsequent increase of activity. Nevertheless substitutions of the phenyl ring **36-39** did not produce any activity increase. Compounds **33**, **34** are the most active compounds in the series of *N*-2-monosubstituted INH derivatives. The activities of INH and compounds **33**, **34** are comparable. It can be assumed due to different log *P* values of these three compounds, that the lipophilicity is not an important parameter for the activity. Structural-similarity but different biological activities of **33** (*n*-C₄H₉) and **32** (CH₃CH=CHCH₂-dehydrogenated analogue of **32**) show, that the double bond in aliphatic chain seems to be very important for the excellent anti-TB activity, as well as phenyl moiety is more advantageous than methyl substitution. It could be concluded, that the activity in this group of INH derivatives is positively influenced by electron-withdrawing lipophilic substituents.

4.3 *N*-2 Disubstituted Hydrazone Derivatives

These compounds are the alkyl analogues derived from **29**. The INH derivative substituted by *iso*-propyl (**42**) or acetyl (**52**) in *N*-2 are the simplest analogues of INH with acceptable anti-TB activity in the *N*(2)-disubstituted series of INH hydrazides. Compounds **42** and **52** represent the models of the following hydrazone INH derivatives (Table 3) and sulphuric isosteres (sulphonamide derivatives) **56**, **57** (Fig. 3). Methyl analogues were showed less activity

than *N*(2)-monosubstituted INH derivatives. Only compound **42** exhibited much higher activity than **29**, comparable with **30**. Phenyl substituted INH derivatives possess higher activity than the compounds substituted by cyclohexane **43**, **44** (hydrogenated analogues).

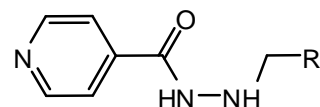


Table 2. The Most Active *N*(2)-Monosubstituted INH Derivatives 29-41, their MIC (μ g/ml)

Comp	R	MIC (μ g/ml)	Comp	R	MIC (μ g/ml)
29	CH ₃	40.0	36	2-F-C ₆ H ₄	0.20
30	CF ₃	0.39	37	3-F-C ₆ H ₄	0.39
31	<i>n</i> -C ₃ H ₇	40.0	38	4-F-C ₆ H ₄	0.39
32	<i>n</i> -C ₄ H ₉	5.0	39	3-CF ₃ -C ₆ H ₄	0.20
33	CH ₃ CH=CHCH ₂	0.02	40	C ₆ H ₅ CONHNHCH ₂	15.0
34	C ₆ H ₅ CH=CH	0.03	41	C ₆ H ₅ CONHNH(CH ₃)CH	15.0
35	C ₆ H ₅	0.20			

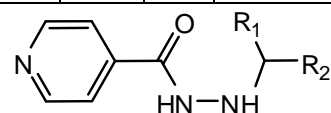
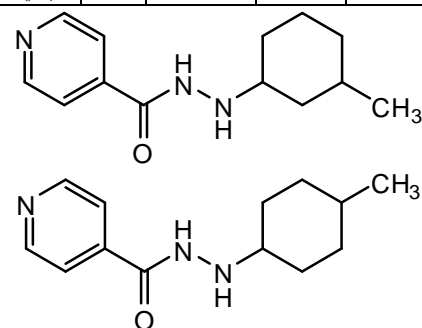
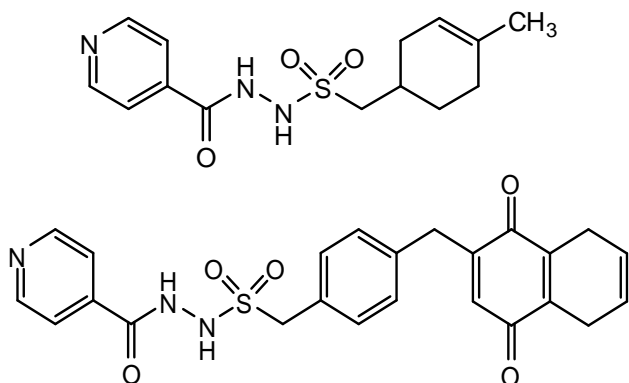


Table 3. The Most Active *N*(2)-Disubstituted INH Derivatives 42 and 45-55, their MIC (μ mol/l).

Comp.	R ₁	R ₂	MIC (μ g/ml).	Comp.	R ₁	R ₂
42	CH ₃	CH ₃	0.4	50	3,4-CH ₃ O-C ₆ H ₃	CH ₃
45	C ₆ H ₅	CH ₃	1.0	51	3,4-Cl-C ₆ H ₃	CH ₃
46	2-F-C ₆ H ₄	CH ₃	0.98	52	O=	CH ₃
47	3-F-C ₆ H ₄	CH ₃	1.6	53	O=	<i>n</i> -C ₈ H ₁₇
48	4-F-C ₆ H ₄	CH ₃	\leq 0.49	54	S=	NH ₂
49	3-CF ₃ -C ₆ H ₄	CH ₃	\leq 0.49	55	OH	CCl ₃





Compound	MIC (µg/ml)	Compound	MIC (µg/ml)
43	4.0	56	10.0
44	5.0	57	0.03

Fig. (3). Compounds **43**, **44** (Klopman, et al., 1996) and **56**, **57**, their MIC (µg/ml).

Halogenation of phenyl seems to be more convenient substitution in the aromatic ring for these hydrazides. Very specific seems to be the activity of acyl derivative **52** and **53** (Suriyati, et al., 2004). While acetyl derivative **52** showed no activity, homologous nonanoyl derivative **53** is one of the most active and the most hydrophobic INH derivatives in this paper. Anti-TB efficiency of this compound probably depends on its lipophilicity, planarity and structure-similarity with mycobacterial membrane composition. These facts probably facilitate the transport of a molecule through the lipophilic membrane into mycobacterial cell. Acyl prodrug is hydrolyzed inside the cell by means of an amidase and INH is released. Both compound **53** (nonanoyl derivative) and compounds **55** (hydroxylic derivative) and **57** (sulphonamide derivative) showed high activity, comparable with INH. It could be only speculated about SARs of both derivatives **55** and **57**. According to the result varieties of the biological activities of **56**, **57** it can be only postulated, that anti-TB activity is independent on the presence of sulphonic moiety (Desai, 1999).

The INH substituted by imidazoline-4-one derivatives **58-62** (Szymanska, and Kiec-Kononowicz, 2002) are structurally interesting compounds derived from substituted guanidine (Fig. 4). This compound type can illustrate nitrogenous isosteres as well as cyclic analogues of dimethyl *N*(2)-disubstituted hydrazide **42** at the same time. Nevertheless contrary to **42** compounds **58-62** show similar lipophilicity as phenyl derivatives, but much less activity, especially dichloro derivatives **61** and **62**. The most promising compound within series 5-(3-chlorobenzylidene)-2-(isonicotinoylhydrazino)-imidazoline-4-one (**59**) with *in vitro* activity comparable with rifampicin (MIC=0.8 µg/ml, SI>78) and acceptable selectivity index SI > 10 was also tested *in vivo* in the

animal TB model but exhibited insignificant activity (Szymanska, and Kiec-Kononowicz, 2002). Carbonyl moiety in imidazoline ring probably causes decrease of activity.

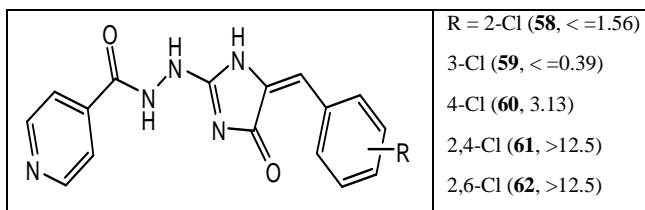


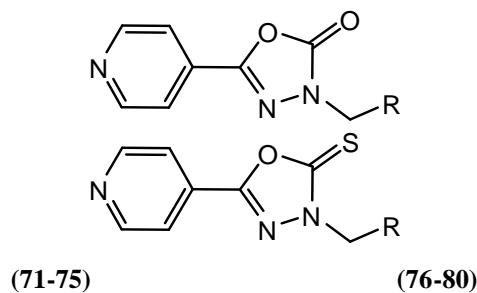
Fig. (4). Compounds **58-62** (48), their MIC (µg/ml).

4.4 Hydrazone Analogues of INH

This chapter involves a great number of INH derivatives. First, various types of cyclic analogues are described and further various *N*(2)-substituted INH hydrazones (Schiff base derivatives) are discussed.

4.4.1*N*-2 Cyclic Analogues of INH

Pyrrole (Arora, et al., 2004) and thiazolidine (Desai, 1999; Vigorita, et al., 1992) derivatives of INH represent the compounds with *N*(2) cycled in 5-membered heterocycle (Fig. 5). All the compounds **63-66** possess high hydrophobicity and medium activity. Sulphuric and especially sulphonic and carbonyl moieties in hydrogenated heterocycles (compounds **67-70**) probably cause activity decrease contrary to pyrrole derivatives **63-66** as described above. Mamolo *et al.* prepared other cyclic INH analogues by means of cyclization of INH with dimethylcarbamoyl chloride (substituted oxadiazolones **71-75**) or using carbon disulfide (substituted oxadiazolothiones **76-80**) (Mamolo, et al., 2005). At the same time hydrazide *N*(2) atom of INH is substituted by various nonaromatic *N*-heterocycles, see Fig. (6). Although oxadiazolones **71-75** showed higher activity than thione isosteres **76-80**, the anti-TB activity of these compounds is low and seems to be independent on substitution of oxadiazole. All these compounds represent structurally similar derivatives as thiazolidines **67-70** discussed above; the same low activity (Fig. 7). Ring-substituted by carbonyl or thiocarbonyl moiety seems to be again disadvantageous for anti-TB activity.



Comp.	R	MIC	Comp.	R	MIC
71		2.5	76		40.0
72		2.5	77		40.0
73		1.25	78		10.0
74		2.5	79		20.0
75		1.25	80		40.0

Fig. (5). Substituted oxadiazolones and their thione derivatives **71-80** (Mamolo, et al., 2005), their MIC ($\mu\text{g/ml}$) are mentioned in brackets.

4.4.2 N-2 Substituted INH Hydrazones

The hydrazones of INH act main role in the research of new anti-TB agents. The substitution can increase lipophilicity that leads to better bioavailability. Various INH modifications are in Fig. 7.

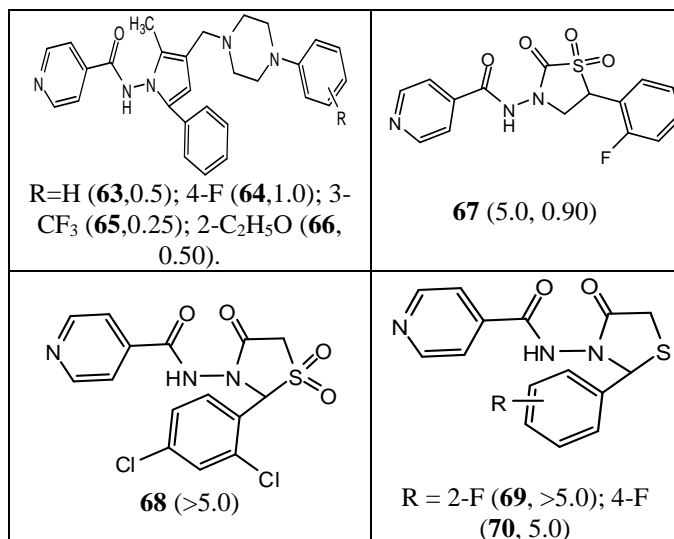


Fig. (6). Pyrrole derivatives **63-66** (Arora, et al., 2004) and thiazolidine derivatives **67-70** (Desai, 1999; Vigorita, et al., 1992), their MIC ($\mu\text{g/ml}$).

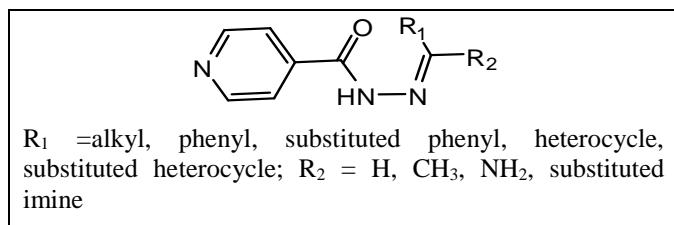


Fig. (7). General structure N(2)-substituted INH

hydrazones.

4.4.3 N-2 Monosubstituted Hydrazones of INH

Most compounds discussed in (Table 4 & Table 5) represent dehydrogenated analogues of N(2)-monosubstituted hydrazide INH derivatives (Table 2). It can be assumed, that these dehydrogenated compounds possess higher lipophilicity; compare the compounds in (Tables 5, 6 with Table 3). The hydrazides **29** and **30** showed significantly less activity than their dehydrogenated analogues **81** and **82**. Similar dependence can be probably observed also at longer alkyl C5-C10 homologues **84-86**. This influence of hydrazone double bond was not confirmed in styryl derivatives **34** and **87**, whose anti-TB activities are similar. Compound **53** possesses similar lipophilicity and anti-TB activity with hydrazone **85**. Structure and property similarities of both compounds probably cause the same mechanism of action. It can be supposed, that the activity does not strictly depend on lipophilicity, see compounds **83-86**. Higher hydrophobicity make transport through mycobacterial wall easier, but too high lipophilicity causes problems with water solubility, see activities of compounds **86**, **88**, **89**. The double bond positively influences the activity due to its easy hydrolysis and fast INH release from discussed molecules. Phenyl substituted hydrazones showed higher activity than the above discussed phenyl substituted hydrazides, although compounds **35** and **91** exhibited similar efficiency.

Compounds **92** (3-F), **94** (3-CF₃), **107** (4-NO₂), **112** (4-(C₂H₅)₂NCH₂NH), **114** (4-CH₃CONH) are the most active. As showed, the activity is directly dependent neither on hydrophobicity, nor on electron parameters. Steric parameters (substituent bulkiness) or substituent position possess probably some influence on anti-TB effect. It is interesting, that methyl derivative **81** exhibited higher activity than phenyl derivative **91** contrary to activities of **29/35**. Anti-TB activity of **91** is comparable with *iso*-propyl derivative **83**. Both compounds possess also similar log *P* values.

It can be assumed according to above presented biological data, that double bond in N(2)-monosubstituted hydrazones of INH (compounds **81-119**) increases anti-TB efficiency due to an easy hydrolysis of this bond and INH release in cell compared with the corresponding discussed hydrazides. Some isonicotinoylhydrazones with different substituents in the benzene ring at the N(2)-position showed superoxide scavenging activity (SSA). This effect was investigated using xantine/xantine oxidase system to generate superoxide anion. It was found that antioxidant action may be responsible for their beneficial effects such as anti-TB activity and lower acute toxicity than INH, like **120**, **121**, Fig. (8). The highest SSA was measured for the compounds possessing hydroxyl group in the *ortho*-position of benzene ring.

Compound **96** showed the most significant antioxidant effect. Subsequent substitution of phenyl ring by ethoxy moiety or chlorine atoms (compounds **120** and **121**) causes antioxidant activity decrease, but increase of anti-TB effect. The compounds without 2-phenolic moiety have lost antioxidative activity. Halogen atoms in *ortho*- or *meta*-position increase anti-TB activity, see compounds **92**, **122** (Georgieva, and Gadjeva. 2002).

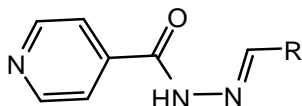


Table 4. The Most Active Aliphatic *N*(2)-Monosubstituted Hydrazones of INH **81-90**, their MIC ($\mu\text{g/ml}$).

Comp.	R	MIC ($\mu\text{g/ml}$)	Comp.	R	MIC ($\mu\text{g/ml}$)
81	CH ₃	0.025	86	<i>n</i> -C ₁₀ H ₂₁	86
82	CF ₃	0.05	87	C ₆ H ₄ CH=CH	87
83	<i>i</i> -C ₃ H ₇	0.1	88	CH ₃ CH=CHC ₇ H ₁₄	88
84	<i>n</i> -C ₅ H ₁₁	0.15	89	CH ₃ (CH ₂) ₇ -CH=CH-(CH ₂) ₇ -CH ₂	89
85	<i>n</i> -C ₆ H ₁₃	0.03	90	HOCH ₂ (CHOH) ₄	90

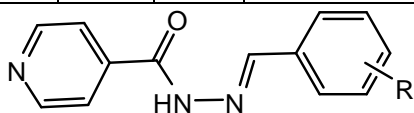


Table 5. The Most Active Aromatic *N*(2)-Monosubstituted Hydrazones of INH **91-117**, their MIC ($\mu\text{g/ml}$).

Comp.	R	MIC ($\mu\text{g/ml}$)	Comp.	R	MIC ($\mu\text{g/ml}$)
91	H	0.1	106	4- <i>i</i> -C ₄ H ₉ O	0.2
92	3-F	0.012	107	4- <i>i</i> -C ₃ H ₁₁ O	0.05
93	4-Cl	0.1	108	2-NO ₂	0.1
94	3-CF ₃	0.05	109	4-NO ₂	0.1
95	4-CF ₃	<0.2	110	4-(CH ₃) ₂ N	1.0
96	2-HO	0.1	111	4-(C ₂ H ₅) ₂ N	0.6
97	3-HO	0.1	112	4-(C ₂ H ₅) ₂ NCH ₂ NH	0.08
98	4-HO	0.1	113	4-CH ₃ COO	1.0
99	4-CH ₃	0.1	114	4-CH ₃ CONH	0.08
100	3-CH ₃ O	0.1	115	4-C ₂ H ₅ SO ₂	0.1
101	4-CH ₃ O	0.1	116	3,4-CH ₃ O	1.0
102	4-C ₂ H ₅ O	0.1	117	3-F-4-CH ₃ O	<0.2
103	4-HOC ₂ H ₅ O	0.1	118	2-HO-4-CH ₃ O	1.0
104	4-C ₃ H ₇ O	1.0	119	4-HO-3-CH ₂ =CHCH ₂	0.1
105	4-C ₄ H ₉ O	1.0			

The phenyl derivative **91** was used as a model for a number of heterocyclic *N*(2)-substituted hydrazones preparation

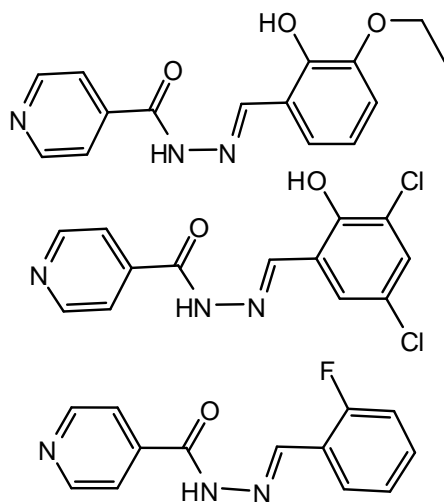
either isosteres or cyclic analogues of **91** (El-Shaer, et al., 1998). The effect of simple heterocyclic derivatives is comparable with phenyl-model compound **91**. More complicated modifications of **91** (piperonyl or 3-formylchromone derivatives **126-128**) showed lower anti-TB activity. Analogue of compound **120**-pyridoxal isonicotinoyl hydrazone **129** (Fig. 9), produced by the Schiff base condensation of pyridoxal and INH has been identified as effective iron chelator *in vitro* and *in vivo* evaluations. Pyridoxal isonicotinoyl hydrazones undergo significant amino acid catalyzed hydrolysis in cell culture medium and in serum, achieving equilibrium with their corresponding aldehydes and hydrazides. Iron is involved in a broad spectrum of essential biological functions such as oxygen transport, electron transfer and DNA synthesis. Compound **129** and all (3-hydroxypyridin-4-ylmethylene) derivatives have become uninteresting as anti-TB agents because of their potential toxicity cause interactions with biological redox systems (Buss, and Ponka. 2003).

4.5 *N*(2)-Disubstituted Hydrazones of INH

N(2)-disubstituted compounds, see Table 6, are more lipophilic than *N*(2)-disubstituted hydrazides (Table 4) or *N*(2)-monosubstituted hydrazones (Tables 4, 5). Methyl or trifluoromethyl moiety represents the second substituent. Activities of some of these compounds are higher than hydrazides but similar to monosubstituted hydrazones. Probably an easier double bond hydrolysis of those hydrazones causes higher efficiency compared with hydrazides. On the other hand branching of chain by various types and bulky substituents protect the hydrazone double bond sterically and make hydrolysis difficult. It can be explanation very low activity of some compounds (Table 6).

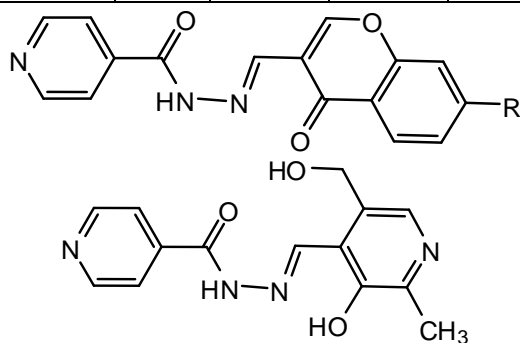
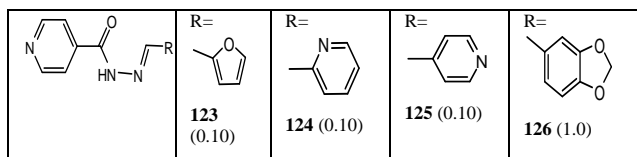
Very interesting activity showed **133** (cyclohexylidene substituent) contrary to **132** (cyclopentylidene substituent) or **134** (phenyl substituent), that possessed significantly low effect. *N*(2)-cyclohexylidene isonicotinic acid hydrazone has exhibited the highest activity from all described INH derivatives. It was chosen for its strong activity (MIC = 0.006 $\mu\text{g/ml}$), low toxicity and excellent bioavailability for exploration. Its calculated log *P* is greater than the calculated log *P* for INH (Hearn, and Cynamon. 2004). The *N*(2)-mono- and disubstituted hydrazones **78/130** and **79/131** and **92/136** showed similar activities. Chlorine and bromine substituted phenyl analogues and disubstituted phenyl analogues possessed no activity compared with *N*(2)-monosubstituted hydrazones. Anti-TB activities of compounds **134** and **146** exhibited very different properties as well. Both compounds differ from each other only by R₂ substituent, **134** (R₂ = CH₃), **146** (R₂ = CF₃), nevertheless the compound **146** showed much less activity than **134**. These facts described in this section can confirm above proposed theory about easy hydrolysis necessity of double

bond in *N*(2) of INH. The compounds **118** or **127** were used as the model compounds for a series of new antituberculotics **148-153** prepared by Shiram *et al.* (Sriram, et al., 2005) (Fig. 9). All the compounds are substituted by phenolic moiety in the C(2) position, whereas in the C(3) position of phenyl different substitutions can be found. Due to 2-phenolic moiety all the compounds show antioxidant activity as discussed above as well as some anti-TB efficiency, which are unfortunately lower than at **118**.



120 (MIC 0.07 μ mol); **121** (MIC 0.07 μ mol); **122** (MIC 0.04 μ mol)

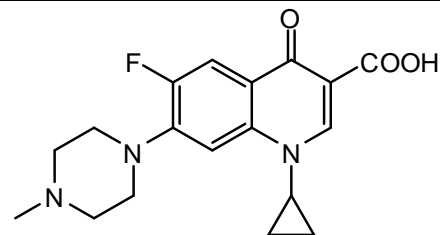
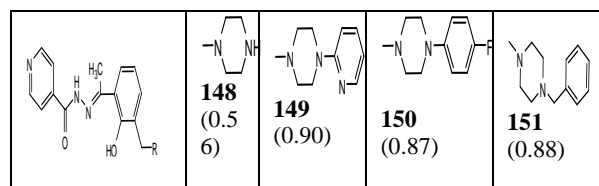
Fig. (8). Substituted phenyl hydrazones of INH **120-122** (Georgieva, and Gadjeva. 2002), their MIC (μ mol).

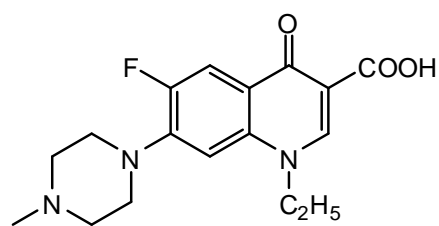


R = CH₃ (**127**, 1.0, 2.33) R=Cl (**128**, 1.0, 2.57)
129

Fig. (9). INH derivatives substituted by heterocycles **123-129** (Klopman, et al., 1996; El-Shaer, et al., 1998), their MIC (μ g/ml).

The adducts of INH with antibacterial fluoroquinolones **152** and **153** exhibited the lowest activity within the series. This fact could not be explained. Sriram *et al.* prepared thioureic derivatives of *N*(2)- disubstituted hydrazones of INH **154-159** (Sriram, et al., 2006). The aim of this work was preparation of adduct anti-TB active thiocarbanilide derivatives with INH. Thioamide (CSNH) moiety is anti-TB very important component and it can be found in clinical practice used drugs ranking to thiocarbanilide group, e.g. ethionamide. These interesting compounds showed comparable activity with INH against *M. tb* H37RV and very high effect against MDR-TB strains (Fig.10). After hydrolysis and probable releasing of INH in mycobacterial cell the activity of INH is potentiated by the second part of molecule with thioureic moiety. The activity within the series of thioureic derivatives is probably dependent on the presence of small lipophilic electron donating substituents in the C(4) position of terminal phenyl ring. Two molecules of INH with pyridine connecting linker **160** (Bottari, et al., 2001) may be a derivative of compound **124** (Fig.11). The dimer **160** showed higher lipophilicity, but lower activity compared with **124**. On the other hand **160** exhibited higher anti-TB efficacy compared with INH dimer **8**. *N*(2)-(1-alkyl-2,3-dihydro-2-oxo-1*H*-3-indolyliden)-4-pyridine carboxylic acid hydrazone derivatives **161-164** were synthesized in a trial to overcome the resistance developed with therapeutic uses of INH. These hydrazones derived from 1-alkylisatin and INH have shown MIC in 10 μ mol/ml against bovine and human sensitive and human resistant strains of *M.tb* (Abou-Fadl, et al., 2003). Further indolyl INH derivatives **165-168** were prepared (Sriram, et al., 2006). All compounds are substituted by anti-TB active gatifloxacin at the same time. These fluoroquinolone INH derivatives showed higher activity than the above discussed quinolone INH derivatives **152** and **153** (Sriram, et al., 2005). All compounds **165-168** are more lipophilic than gatifloxacin, which is important for penetration of these compounds through bacterial/mycobacterial cell. Assuming, that the issue of penetration is even more crucial for quinolone activity against *Mycobacteria* sp.





152 (1.30)

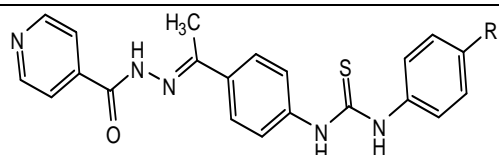
153 (1.26)

Fig. (10). INH derivatives substituted by heterocycles **148-153** (Sriram, et al., 2005), their MIC (μmol) are mentioned in brackets.

All compounds were tested against *Mtb*, and against MDR-TB and they showed similar activity. According to the results, see Fig. (12), it can be assumed, that unsubstituted gatifloxacin exhibited higher activity against *Mtb*, whereas prepared analogues **165-168** showed good efficacy against MDR-TB strains. All the compounds were also evaluated for their cytotoxicity and all the discussed derivatives were found to be non-toxic until 62.5 $\mu\text{g/ml}$.

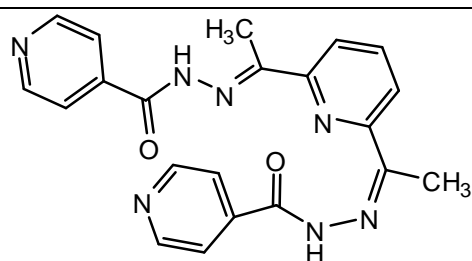
4.6 Aminohydrazones

Aminohydrazone derivatives represent *N*(2)-disubstituted hydrazones of INH, where one of the substituents is amino or imino moiety. Structure of aminohydrazones is isosteric to *N*(2) methyl and phenyl disubstituted hydrazones. Some of these compounds, see Fig. (13), were described as *in vitro* anti-TB active and some of them exhibited an activity toward a human strain of *M. avium* resistant to the primary drugs INH, rifampicin and ofloxacin (Mamoto, et al., 1996). A number of various substituted 2-amino-2-(isonicotinoylhydrazono) ethyl aryl ethers **169-180** were tested and some of them possessed anti-TB activity. Unfortunately the results of the majority of aminohydrazones showed, that amino or substituted imino moieties did not produce any highly active compound, nevertheless they proved other interesting properties, and seem to be perspective group in the future as INH activity scale compounds.

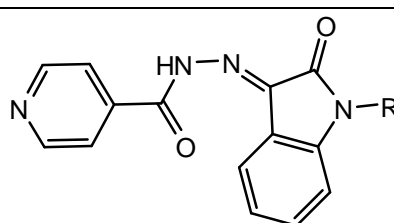


R = H (**154**, 4.00, 4.00); CH₃ (**155**, 1.93, 3.86)
 Br (**156**, 1.66, 1.66); F (**157**, 0.49, 0.49)
 OCH₃ (**158**, 0.47, 1.85); OC₂H₅ (**159**, 0.89, 1.79)

Fig. (11). Thioureic INH derivatives **154-159** (Sriram, et al., 2006), their MIC (μmol) as against *M. tb* H37RV, as against MDR-TB are mentioned in brackets. The activity of INH was 1.46 μmol , or 90.94 μmol .

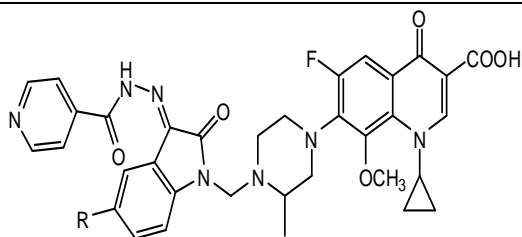


160 (0.39, 2.56)



R = H (**161**)
n-C₃H₇ (**162**)
 CH₂C₆H₅ (**163**)
 C C-CH₃ (**164**)

Fig. (12). Simple INH derivatives substituted by heterocycles **160** (Bottari, et al., 2001) and **161-164** (Abou-Fadl, et al., 2003), their MIC ($\mu\text{g/ml}$).



R = F (**165**, 0.78, 0.78)
 CH₃ (**166**, 0.78, 0.78)
 Cl (**167**, 0.39, 0.78)
 H (**168**, 0.20, 0.78)

Fig. (13). Indolyl-gatifloxacin-INH derivatives **165-168** (Sriram, et al., 2006), their MIC ($\mu\text{g/ml}$) against *Mtb*, and against MDR-TB strains are mentioned in brackets. The activity of gatifloxacin was 0.20 $\mu\text{g/ml}$, or 3.12 $\mu\text{g/ml}$.

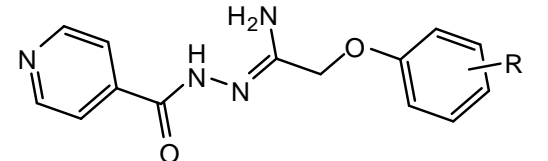
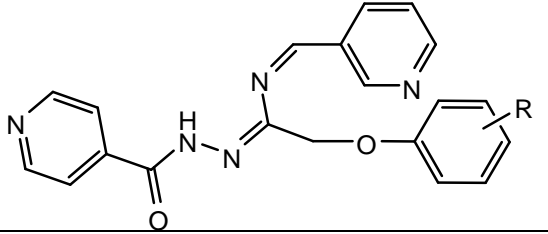
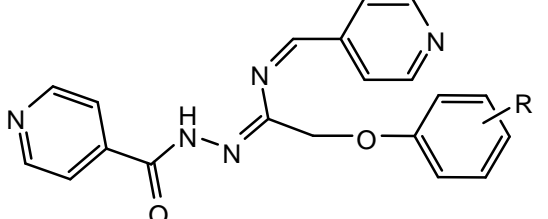
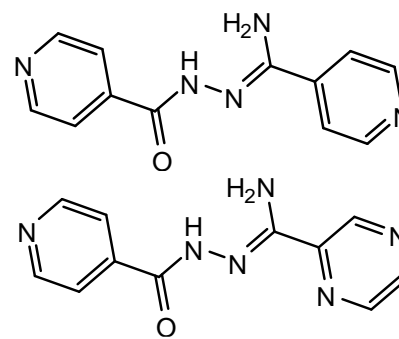
	R = 2-CH ₃ (169 , 12.2, 1.29); 3-Cl (170 , 3.12, 1.51); 4-Cl (171 , 3.12, 1.52); 4-NO ₂ (172 , 3.12, 0.46); 4-CH ₃ CONH (173 , 50.0, 0.90)
	R = H (174 , 25.0, 2.73); 4-Cl (175 , 50.0, 3.13) 4-NO ₂ (176 , 50.0, 2.26)
	R = 3-CH ₃ (177 , 50.0, 3.21); 4-CH ₃ O (178 , 50.0, 3.01); 3-Cl (179 , 50.0, 3.20); 4-Cl (180 , 50.0, 3.15)

Fig. (14). The most active amino substituted *N*(2)-disubstituted INH hydrazones **169-180** (Cocco, et al., 1999; De Logu, et al., 2002), their MIC ($\mu\text{g/ml}$).

Compounds **170** (3-Cl), **171** (4-Cl) and **172** (4-NO₂) were the most effective against MDR-TB strains (Cocco, et al., 1999). Four compounds **169-172** were additionally evaluated also against 16 strains of *Mtb* isolated from clinical specimens and five reference strains, including four MDR strains. They were significantly more effective than INH against INH resistant *M.tb* ATCC 35822 (MIC 200 mg/ml). Lipophilicity of aminohydrazones was not the critical factor affecting their potency. Their combinations with INH, *para* aminosalicylic acid, rifampicin and ethambutol have shown synergic interaction. The ability of these compounds to inhibit specifically the growth of *Mtb*, the high selectivity index and their ability to enhance the activity of standard drugs is promising for future drug development (De Logu, et al., 2002).

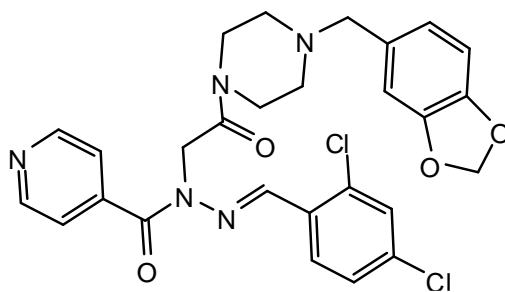


181

182

Fig. (15). Prepared amidines as prodrugs INH (Glushkov, et al., 2004).

Fig. (15) illustrates INH derivatives **181** and **182** (isosteres of compound **125**), which were prepared as prodrugs



R = **183** (0.25 - 1.0)

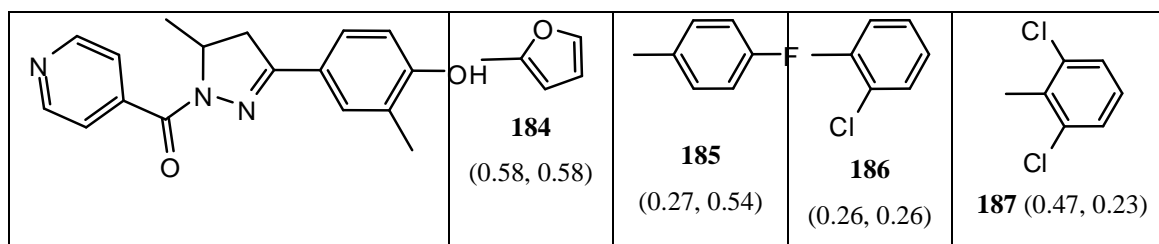


Fig. (16). The most active *N*(1),*N*(2)-disubstituted hydrazones of INH: compound **183** (Sinha, et al., 2005), their MIC ($\mu\text{g/ml}$) are mentioned in bracket, and compounds **184-187** (Shaharyar, et al., 2006), their MIC (μM) against *Mtb*, and against INH-resistant *Mtb* are mentioned in brackets.

(Glushkov, et al., 2004). These amidines possess higher lipophilicity than INH. Their penetration through the mycobacterial membrane should be easier. Activities of both compounds depend on hydrolysis reaction rate. Compound **181** has shown neither antioxidative nor iron-chelating activities due to lack of hydroxylic moiety in C'(3) of pyridine ring.

4.7 Hydrazones of *N*-1,*N*-2-Substituted of INH

A series of isonicotinic acid *N*(2)-arylidene-*N*(1)-(2-oxo-2-(4-aryl-piperazin-1-yl)-ethyl)-hydrazide derivatives were

prepared as new anti-TB agents. Compound **183**, see Fig. (16), possessing 2-oxo-2-(4-(piperonyl)-piperazin-1-yl)-ethyl- residue at *N*(1) position of INH was identified as the most active compound with the *in vitro* anti-TB activity (MIC 0.25-1.0 $\mu\text{g/ml}$) against *Mtb H37Rv* and ATCC 27294 strain and clinical isolates (sensitive strains) of *Mtb*. It has shown also good potency against MDR-TB strains (Sinha, et al., 2005). Compound **183** composes from combinations of anti-TB active fragments, which can be found in compounds **91** (phenyl), **126** (piperonyl), **151** (piperazinyl). This compound showed a higher activity than the above mentioned *N*(2)-monosubstituted hydrazones of INH. Further *N*(1),*N*(2)-disubstituted hydrazones of INH (Shaharyar, et al., 2006), both INH nitrogens are incorporated in a substituted pyrazoline moiety. These derivatives **184-187** are isosteres of pyrrole INH derivatives **63-66** (Arora, et al., 2004), respectively cyclic analogues of oxadiazolones **71-75** and oxadiazolo-thiones **76-80** prepared (Mamolo, et al., 2005). All the compounds showed very interesting activities not only against *Mtb*, but also against INH resistant strains, Fig. 16. The compounds

Table 6. The Most Active *N*-2-Disubstituted Hydrazones of INH **130-147**, their MIC ($\mu\text{g/ml}$)

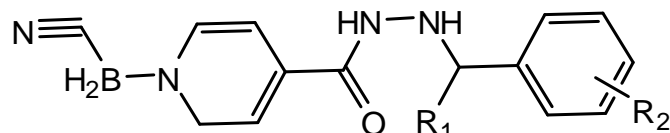
Comp.	R1	R2	MIC ($\mu\text{g/ml}$)	Comp.	R1	R2
130	CH ₃	CH ₃	0.02	139	3-Br-C ₆ H ₄	CH ₃
131	CF ₃	CH ₃	0.05	140	3-CF ₃ -C ₆ H ₄	CH ₃
132	(CH ₂) ₄	---	0.1	141	2,4-Cl-C ₆ H ₄	CH ₃
133	(CH ₂) ₅	----	0.006	142	3,4-Cl-C ₆ H ₃	CH ₃
134	C ₆ H ₅	CH ₃	0.1	143	2,4-OCH ₃ -C ₆ H ₃	CH ₃

with halogen substituted phenyl group showed more activity than the compound **184** substituted with furyl moiety. This is the same trend as for pyrrole INH derivative **65**. Compounds **185**, **186** were found to be most active within this series and were more active than INH. These compounds were evaluated for toxicity (MTT assay) and they were found to be nontoxic till 62.5 $\mu\text{g/ml}$.

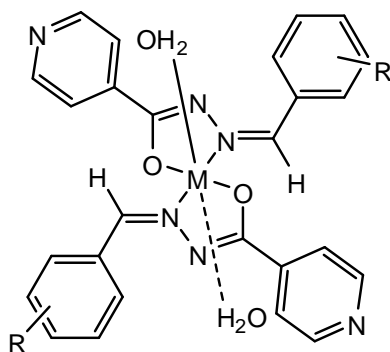
4.8 Chelates Analogues of INH

Preparation of metal complexes derived from various biologically active compounds has become very popular for some years. This way of several INH chelates of the general structure **188** were prepared and tested for their anti-TB activity. Some of them showed very interesting activities against MDR-TB strains and non-tubercular *Mycobacteria* (NTM). Copper(II), nickel(II) and cobalt(II) chelates (MeL2 (H₂O)₂) of isonicotinoyl hydrazones (ISNEs) obtained from INH and fluorinated carbonyl compounds were investigated, Fig. 17. These chelates were prepared from active *N*-2-monosubstituted hydrazones **92**, **94** and **117**. The ligands were effective against *Mtb H37Rv* and also some extent, *M. avium* complex. The activity of these complexes should be prolonged due to the slow release of active ligands, while metal ion should play a role connected with the enhanced capability to cross the *Mtb* wall (Bottari, et al., 2000). Octahedral cobalt (II) complexes of isonicotinoylhydrazones exhibited the highest MIC values of activity ranging from 0.1 to 0.39 $\mu\text{g/ml}$ (Bottari, et al., 2001), nevertheless these activities are similar with antituberculous effects of **92**, **94** and **117**. Unfortunately no toxicological results of these chelates were found. The metalchelates have a polar core consisting of the metal ion

135	2-F-C ₆ H ₄	CH ₃	0.2	144	3,4-OCH ₃ -C ₆ H ₃	CH ₃
136	3-F-C ₆ H ₄	CH ₃	<0.05	145	3-F-4-OCH ₃ -C ₆ H ₃	CH ₃
137	4-F-C ₆ H ₄	CH ₃	0.05	146	C ₆ H ₅	CF ₃
138	3-Cl-C ₆ H ₄	CH ₃	>20	147	COOH-(CH ₂) ₂	CH ₃



and the electronegative atoms of ISNEs involved in coordination, surrounded by the hydrophobic moieties of the ligands, which form an outer lipophilic envelope. Such an arrangement should facilitate the diffusion through biomembranes, thus enhancing the anti-TB effectiveness of ISNEs. This metalchelates could act as repositories for the active ligands and could thus be potentially longer-acting anti-TB agents. They possessed also moderate activity against *M. avium* complex (Maccari, et al., 2004).



M = Cu²⁺, Ni²⁺, Co²⁺ R = 3-F, 3-CF₃, 3-F-4-CH₃O **188**

Fig. (17). The structure of the most effective isonicotinoylhydrazone chelates of the general structure **188** tested for their anti-TB activity.

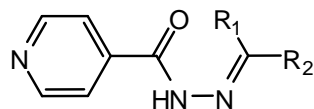


Table 7. The Most Active Cyanoborane Adducts of INH **189-195**, their MIC ($\mu\text{g/ml}$).

Comp.	R1	R2	MIC ($\mu\text{g/ml}$)	Comp.	R1	R2
189	H	H	0.80	193	H	3,4-CH ₃ O
190	H	2-F	3.13	194	CH ₃	H
191	H	3-F	0.80	195	CH ₃	3,4-Cl
192	H	3-	0.80			

		CF ₃			
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Table 8. The Selected Anti-TB Active INH Derivatives compared with Standards (Isoniazide =INH, Pyrazinamide = PZA, Ciprofloxacin = CPF) (Imramovsky, et al., 2007)

Compd.	MIC ($\mu\text{g/ml}$)	MIC ($\mu\text{mol/l}$)			
	<i>M. tbc</i> H37Rv	<i>M. tbc</i> 331/88	<i>M. avium</i> 330/88	<i>M. kansasii</i> 235/80	<i>M. kansasii</i> 6509/96
197	-	2.0	125	2.0	2.0
198	0.39	4.0	125	500	125
199	0.39	-	--	-	-
200	0.10	4.0	125	500	8.0
INH	0.025–0.20	1.0	>250	>250	>2.0
PZA	6.0–60.0	8.0	>128 -	>128 -	-
CPF	2.0	-	--	-	-

4.9 Cyanoborane Analogues of INH

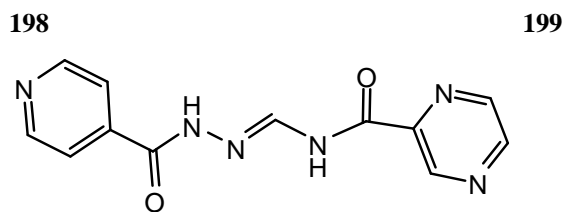
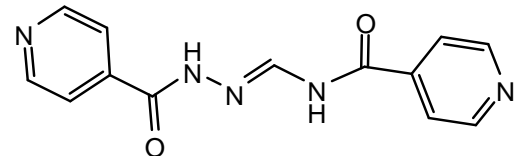
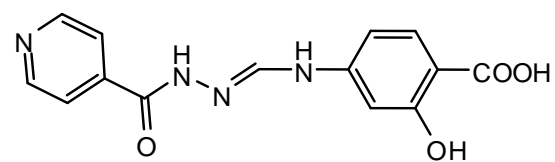
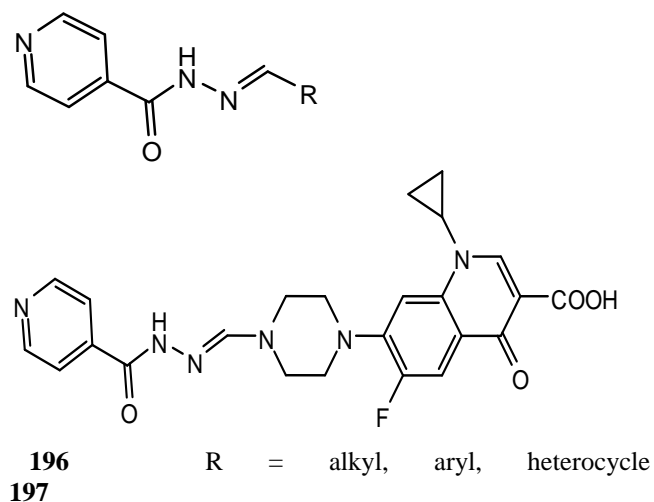
Cyanoborane adducts/salts **189-195** of isonicotinoyl hydrazides were tested for *in vitro* anti-TB activity (Maccari, et al., 2002). The most active cyanoborane analogues were derived from the above discussed *N*-2-mono- and disubstituted INH hydrazide like **35-37**, **45** and **51** and are showed in Table 8. All these derivatives possess a higher lipophilicity than the model compounds, but lower anti-TB activity. On the other hand, some discussed cyanoborane derivatives proved to be more effective anti-TB agents than parent INH in a TB-infected macrophage model (Maccari, et al., 2005).

5 Isoniazide antituberculosic agents

Research on potential anti-TB drugs, especially pyrazine-2-carboxylic acid derivatives (Jampilek, et al., 2005; Dolezal, et al., 2006), was performed. At the beginning of the 21st century, the research was also focused on anti-TB agents derived from INH. A number of INH derivatives of the general structure **196** as prodrug forms have been designed and synthesized in the quest for biologically more potent anti-TB compounds (Imramovsky, and Polanc. 2005; Imramovsky, et al., 2005). Combination of INH with other

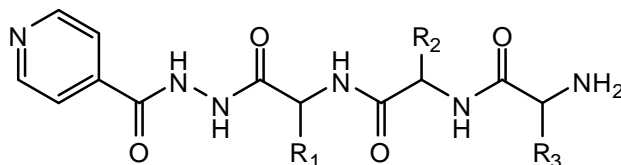
appropriate antibacterial component leads to the prodrugs with prolonged release and possible synergism as e.g. compounds **197-200** (Imramovsky, et al., 2006). Excellent activities of these compounds are shown in Table 8. Their activities are comparable with INH not only against *Mtb* H37Rv, but they exhibited activity also against some clinical isolated atypical strains (Imramovsky, et al., 2007; Imramovsky, et al., 2006; Jampilek, et al., 2006). Adduct of INH with pyrazinamide **200** and dimer of INH **199** are very interesting compounds. The late derivative is methylene homologue of compound **8**. The described compounds are non-toxic, Table 8.

The amino acid and peptide derivatives of general formula **201**, in Fig. (19) as new prodrug forms. Several amino acids (Gly, Ala, Leu, Pro, Phe, Tyr, Arg, Asn) were used to monitor the best bioavailable one, three dipeptides (Ala-Ala, Gly-Gly, Leu-Ala) and two tripeptides (Gly-Gly-Gly, Ala-Ala-Ala) for enzyme hydrolysis release. The results of this study are being investigated (Jampilek, et al., 2006).



200

Fig. (18). The general structure **196** of prepared compounds and some antituberculous effective prodrug of INH derivatives **197-200** (Imramovsky, et al., 2006; Imramovsky, et al., 2007).



201

Fig. (19). The general structure of INH peptide derivatives **201** as new INH prodrug forms prepared as potential anti-TB agents.

The different *N'*-(disubstitutedbenzoyl) Isoniazid derivatives have been obtained. Appropriately, substituted carboxylic acid chlorides were reacted with INH to furnish the *N'*-(disubstitutedbenzoyl) isoniazid derivatives (**202-209**).

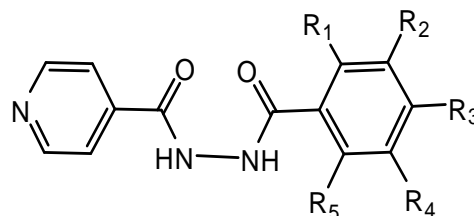


Fig. (20). Structures of first line drugs used in TB treatment, Compounds **202-209**.

Table 9. *In Vitro* Activity of Compounds 9-16 against *M. Tuberculosis* H37Rv Strain, susceptible both to Rifampin and Isoniazid)

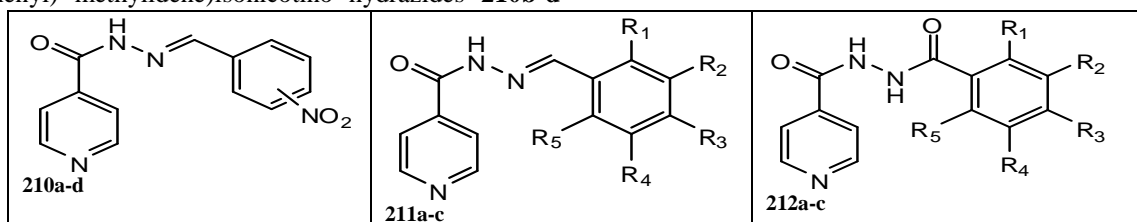
Compound	Compound	MIC (µg/mL)	Compound	Compound	MIC (µg/mL)
202	R ₁ = R ₅ = Cl; R ₂ = R ₃ = R ₄ = H	15.0	206	R ₁ = Cl; R ₂ = R ₃ = F; R ₄ = R ₅ = H	10.0
203	R ₁ = R ₂ = Cl; R ₃ = R ₄ = R ₅ = H	15.0	207	R ₂ = R ₄ = NO ₂ ; R ₁ = R ₃ = R ₅ = H	2.0
204	R ₁ = R ₃ = Cl; R ₂ = R ₄ = R ₅ = H	15.0	208	R ₂ = NO ₂ ; R ₃ = Cl; R ₁ = R ₄ = R ₅ = H	0.5

205	R ₁ = R ₃ = F; R ₂ = R ₄ = R ₅ = H	10.0	209	R ₁ = NO ₂ ; R ₄ = Cl; R ₂ = R ₃ = R ₅ = H	0.5
INH		0.2	RIF		1.0

Compounds **208** and **209** exhibited a significant activity (0.5 µg/mL) when compared with first line drugs as isoniazid (INH) and rifampin (RIF). It suggests that they may be selectively targeted to *Mtb* growth, also considering that they were not cytotoxic to host cells at the same concentration and could be a good start point to find new lead compounds. More information about structure-activity relationship and their *in vivo* antibacterial activity test are in progress. Compounds 15 and 16 exhibited a significant activity (0.5 µg/mL) when compared with first line drugs such as isoniazid (INH) and rifampicin (RIP) and could be a good starting point to develop new lead compounds in the fight against MDR-TB (Neves et al., 2006).

6 Nitroisoniazid derivatives

The *N*'-((*E*)-(nitrophenyl)methylidene) isonicotino hydrazides **210b-d**. These compounds were evaluated for their *in vitro* antibacterial activity against *Mtb* H37Rv using the Alamar Blue susceptibility test and the activity expressed as the MIC in µg/mL (**Table 10**). Depending on the position of the nitro group in the aromatic ring, different MIC values were obtained when compared with phenyl group, demonstrating the importance of the nitro group for the biological activity in this class of compounds. In *ortho* or *meta* position, MIC values were higher (5.0 µg/mL) when compared with the phenyl system (3.2 µg/mL). However, when the nitro group was placed in the *para* position it increases by three folds the biological activity, with MIC values of 1.2 µg/mL. It can be observed some modifications in the anti-TB activity when *N*'-((*E*)-(nitrophenyl) methylidene)isonicotino hydrazides **210b-d**



The anti-TB activities of compounds **210a-d**, **211a-c** and **212a-c** were assessed against *Mtb* using the micro plate Alamar Blue assay (MABA). The final drug concentrations tested were 0.01 to 20.0 µL/mL. none of the nicotinic derivatives tested were active against the *Mtb*. In the case of the INH derivatives containing nitro groups evaluated, the best result was the compound **210d** (1.2 µg/mL) when compared with first line drugs as INH and rifampin (RIP).

were compared with the *N*'-((*E*)-(disubstituted-phenyl)methylidene) isonicotino-hydrazides **211a-c** and *N*'-(disubstituted benzoyl) isoniazid **212a-c** derivatives containing nitro groups. For example, the *N*'-(disubstituted benzoyl) isoniazid **212a-c** were more activity when compared with isonicotino-hydrazides **210b-d** and **211a-c**. The dinitro compound **211a** and the nitrochloro compound **211b** did not show increased anti-TB activity when compared with the mononitroiso-nicotinohydrazide **210d**. However, the compound **211c**, which has respectively, the nitro group and chloro atom at 2 and 3 positions in the aromatic ring presented increased anti-tubercular activity, when compared with the mononitroisonicotino-hydrazide **10b** indicating that chloro atom at 3 position is important for the biological activity (Lourenco et al., 2007).

Table 10. Antimycobacterial activities derivatives **10a-d**, **11a-c** and **12a-c**

Comp.	R	MIC (µg/mL)	Comp.	R	MIC (µg/mL)
210a	Phenyl	3.12	211b	R ¹ = NO ₂ ; R ⁴ = Cl	5.00
210b	o-nitro	5.00	211c	R ² = NO ₂ ; R ³ = Cl	1.20
210c	m-nitro	5.00	212a	R ² = NO ₂ ; R ⁴ = NO ₂	2.00
210d	p-nitro	1.20	212b	R ¹ = NO ₂ ; R ⁴ = Cl	0.50
211a	R ¹ = NO ₂ ; R ³ = NO ₂	5.00	212c	R ² = NO ₂ ; R ³ = Cl	0.50
RIP		1.0	INH		0.2

It suggests that this class of compounds may be selectively targeted to *Mtb* growth, also considering that they were not cytotoxic to host cells at the same concentration and could be a good starting point to find new lead compounds.

7 Discussion and Conclusion

Isoniazid (INH) is a small and very hydrophilic molecule. Most of the compounds possess higher lipophilicity than INH (Imramovsky, et al., 2007; Imramovsky, et al., 2006; Jampilek, et al., 2006). Various INH structure

modifications were discussed. It was shown, that substitution in the C-2 position of pyridine ring as well as quaternisation of pyridine nitrogen is disadvantageous and these facts cause the activity decrease. Most compounds pertain to hydrazide or hydrazone compound family, therefore the structure-anti-TB activity relationship of both compound types have been investigated well. Evaluations of the hydrazide or hydrazone structures were analysed (Klopman, et al., 1996). The results of lipophilicity-activity relationships and SARs, that the discussed lipophilic INH derivatives may possess more significant transport of a molecule through mycobacterial membranes due to higher lipophilicity. The higher anti-TB activity of INH derivatives compared with INH is dependent on the stability of the whole molecule/complex. The double bond in the discussed hydrazones is better/more easily hydrolysed than the single bond in corresponding hydrazides or another *N*(2)-substituted INH derivatives (Imramovsky, et al., 2005; Imramovsky, et al., 2006). It can be concluded, that the molecule INH and its anti-TB activity is structure-specific.

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